thoroughly with water and then dried at 100° (0.1 mm) in a drying pistol containing powdered KOH in order to remove the last traces of formic acid, uv (0.1 N NaOH) 232 nm ($\epsilon$ 31,820), 285 (38,340), 428 (13,530).

*Anal.* Calcd for $C_{14}H_9N_5O \cdot 0.25H_2O$: C, 62.79; H, 3.57; N, 26.15. Found: C, 62.80; H, 3.37; N, 26.08.

**Registry No.**—**1**, 4215-03-6; **3**, 50803-83-3; **5**, 50803-84-4.

### References and Notes

(1) This investigation was supported in part by Research Contract DADA-17-71-C-1001 from the U. S. Army Research and Development Command, Office of the Surgeon General, and by Research Grant C6516 from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service. This is publication no. 1198 from the U.S. Army Research Program on Malaria.
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(12) The similar gradual appearance of an AB pattern indicative of para substitution was noted previously[3] with 2-amino-7-phenethylpteridin-4(3*H*)-one and 2-amino-7-(3-phenylpropyl)pteridin-4(3*H*)-one, neither of which would be anticipated to undergo cyclization if the mechanism in Scheme I is valid.
(13) We are grateful to one of the referees for pointing out that the creation of an electrophilic center at C-7 on protonation is akin to the effect of an *N*-oxide. A convenient method has been reported recently for the direct synthesis of pteridine 8-oxides: H. Yamamoto, W. Hutzenlaub, and W. Pfleiderer, *Chem. Ber.*, **106**, 3175 (1973).

# Nucleotides. II. Syntheses and Deblocking of 1-Oxido-2-pyridylmethyl Protected Nucleosides and Nucleotides[1,2]

Yoshihisa Mizuno,* Takeshi Endo, Teiji Miyaoka, and Kazuyoshi Ikeda

*Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan*

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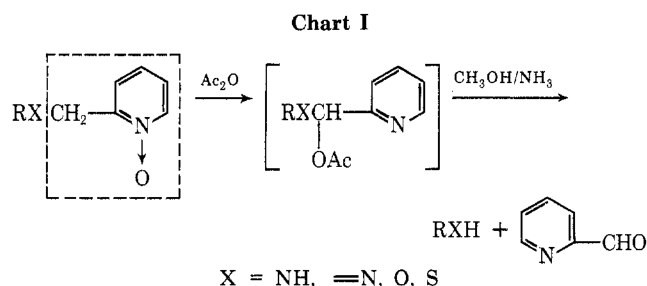
1-Oxido-2-pyridylmethyl group (op group) was found to be useful for protection of amino or hydroxyl groups of adenine, nucleosides (cytidine and adenosine), or phosphate functions of nucleotides (uridine 5′-phosphate and adenosine 5′-phosphate). $N^6$-(1-Oxido-2-pyridylmethyl)adenine (**1**) was prepared by the reaction of 1-oxido-2-pyridylmethylamine (**9**) and 6-methylsulfonylpurine (**11**). $N^4$-(1-Oxido-2-pyridylmethyl)cytidine (**2**) and $N^6$-(1-oxido-2-pyridylmethyl)adenosine (**3**) were also prepared by the reactions of **9** and appropriate sulfonate or sulfone derivatives of nucleosides **8** and **13**. 1-Oxido-2-pyridylmethyl nucleoside 5′-phosphates (**4**, **5**, and **6**) were prepared in excellent yields by the reactions of the nucleotides with 1-oxido-2-pyridyldiazomethane (**15**), a water-soluble alkylating agent newly developed for the present investigation. By the use of **15** op protection could be introduced into phosphate functions of nucleotides in aqueous solution in excellent yields. Deblocking of these op-protected nucleoside (**2**) and nucleotides (**4** and **6**) could be achieved in satisfactory yields (86–96%) by treatment with acetic anhydride, followed by methanolic ammonia.

In the past few years, the development of procedures for the chemical synthesis of oligonucleotides has depended to a significant extent on the design of a new protecting group with specific properties.[3]

In the preceding paper it was shown that 1-oxido-2-pyridylmethyl group (op group)[4] was useful as an easily removable blocking group for amino, imino, and hydroxyl functions[5] (Chart I).

**Chart I**




The present paper deals firstly with the preparation of 1-oxido-2-pyridylmethyl protected nucleosides **2** and **3** (Chart II) as well as 1-oxido-2-pyridylmethyl protected adenine (**1**), secondly with the preparation of the nucleotide derivatives **4**, **5**, and **6** by the use of 1-oxido-2-pyridyl diazomethane (**15**), and finally with the deblocking of these compounds (**2**, **5**, and **6**) with acetic anhydride treatment and subsequent hydrolysis.

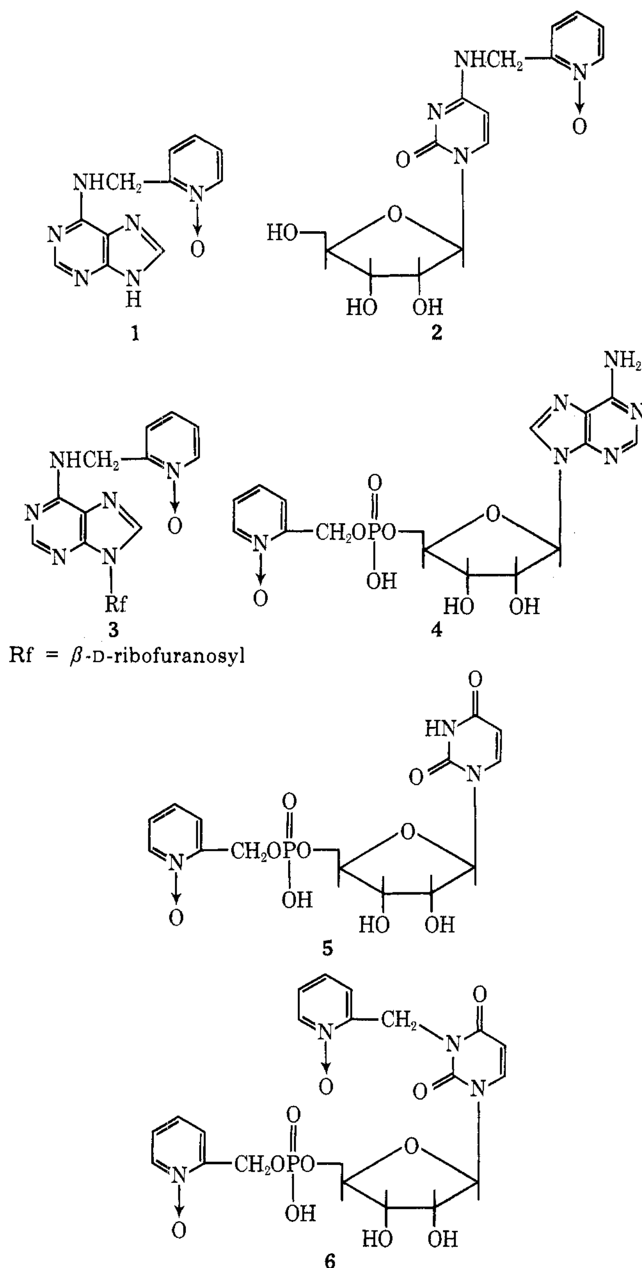
Although two op-protected nucleosides (**2** and **3**) might be prepared by Dimroth rearrangement[6] of the respective 1- or 3-op-substituted nucleosides, we have adopted alternative routes (see Chart III).

Oxidation of 4-thiouridine (**7**)[7] with potassium permanganate (at 0° for 15 min) afforded the corresponding 4-sulfonate (**8**).[8] Without isolation, the reaction mixture was treated with 1-oxido-2-pyridylmethylamine (**9**) at room temperature for 25 hr to give the expected $N^4$-(1-oxido-2-pyridylmethyl)cytidine (**2**) (crude yield was almost quantitative) which was purified by charcoal treatment. The product was homogeneous on tlc and paper chromatography.

The structural confirmation of **2** rests upon the elemental analysis and spectral data (uv, ir, and nmr). Although the isolated yield was rather poor (34.7%), the possibility of optimizing isolation (charcoal treatment) conditions could improve the yield.

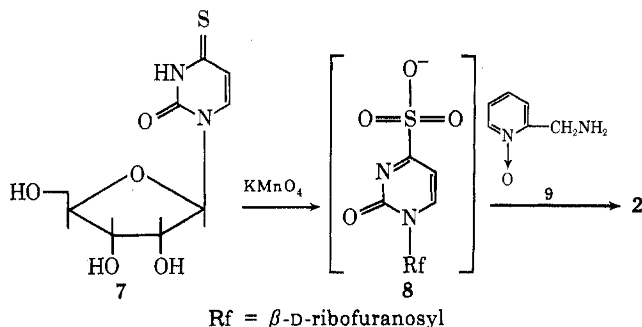
$N^6$-(1-Oxido-2-pyridylmethyl)adenine (**1**) was prepared according to a route shown in Chart IV. The synthetic sequence starts with 6-methylthiopurine (**10**),[9] which on oxidation with aqueous bromine solution afforded the corresponding 6-methylsulfonylpurine (**11**), contaminated with a small amount of 6-methylsulfinylpurine. Without purification, the mixture was treated with 1 equiv of 1-oxido-2-pyridylmethylamine (**9**) to yield **1** in 20% yield. The structure was confirmed by elemental analyses as well as spectral data.

**Chart II**




Treatment of 6-ethylthiopurine-9-(β-D-ribofuranosyl)purine (12) with aqueous bromine solution afforded 6-sulfonylpurine ribonucleoside (13), which was then treated with 1 equiv of 9 to give $N^6$-(1-oxido-2-pyridylmethyl)adenosine (3). The product 3 was purified by silica gel chromatography. Structural confirmation comes from spectral (uv and ir) data and the fact that acid hydrolysis of 3 afforded the $N^6$-op-adenine 1.

**Chart III**




For the preparation of 4-6, a new water-soluble alkylating agent, 1-oxido-2-pyridyldiazomethane (15), was introduced.

It is well established that diazomethane, the parent of the diazoalkanes, is one of the most versatile and useful reagents in organic chemistry. As a methylating agent of reasonably acidic substances, diazomethane has ideal properties. Methyl group is, however, of no use as a protecting group, because of difficulties encountered in its removal.

The synthetic sequence of 15 starts with 2-formylpyridine 1-oxide,[10] which was converted into the corresponding *p*-tosylhydrazone (14) (Chart V) in 79% yield, which in turn was treated with 1 equiv of sodium ethoxide in ethanol at 50°. After work-up, compound 15 was obtained in a yield of 47% as a chloroform solution which contained a small amount of 2-ethoxymethylpyridine 1-oxide (*ca.* 10%) as a by-product. The chloroform solution was colored; ir absorption maxima appeared at 2080 ($N{\equiv}N^+$) and 1235 $cm^{-1}$ ($N{\rightarrow}O$). This solution was employed for the subsequent experiment.

The reaction of the chloroform solution of 15 with acetic or benzoic acid took place rapidly at room temperature with evolution of nitrogen to give 1-oxido-2-pyridylmethyl acetate, mp 67-68°, or benzoate, mp 125-126°, respectively. Yields of the acetate or the benzoate were almost quantitative.

Treatment of an aqueous solution of adenosine 5′-phosphate (16) with the chloroform solution of 15 at room temperature afforded the corresponding 1-oxido-2-pyridylmethyladenosine 5′-phosphate (4) (Chart VI). The product was purified by DEAE-cellulose column chromatography. Hydrolysis of 4 with snake venom phosphodiesterase afforded adenosine 5′-phosphate (16) and 1-oxido-2-pyridylcarbinol (17) in a molar ratio of 1:1. The yield of 4 was 90%.

**Chart IV**

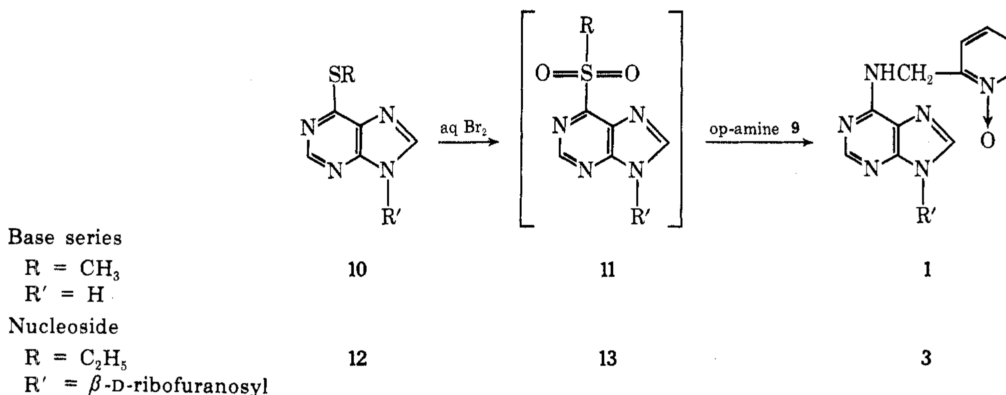


**Chart V**

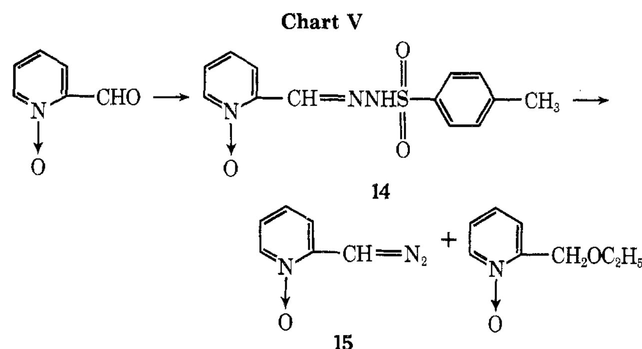
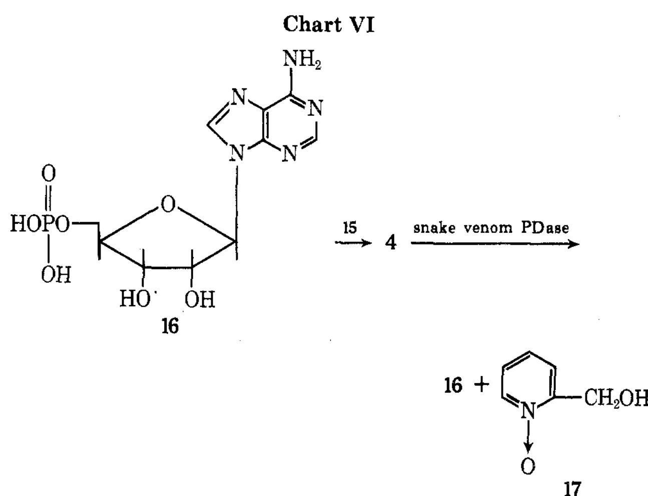
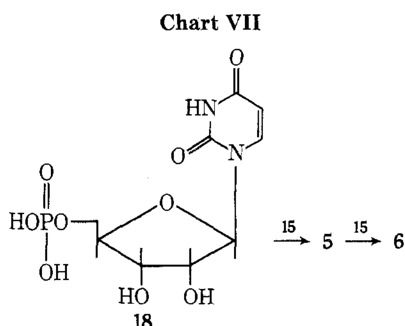



**Chart VI**




On treatment of uridine 5′-phosphate (18) with the reagent 15 at 20° for 30 min, 1-oxido-2-pyridylmethyluridine 5′-phosphate (5) was obtained in 89% yield (Chart VII). The structure of 5 was confirmed by the enzymatic hydrolysis (snake venom phosphodiesterase) to 1-oxido-2-pyridylcarbinol (17) and 18 (Chart VIII) and by the comparison of its spectral properties and electrophoretic mobilities with those of an authentic sample which had been prepared by a general procedure including deacetylation from 2′,3′-di-*O*-acetyluridine 5′-phosphate (19)[13] and 17 by the use of 2,4,6-triisopropylbenzene sulfonyl chloride (TPS) as a condensing agent.

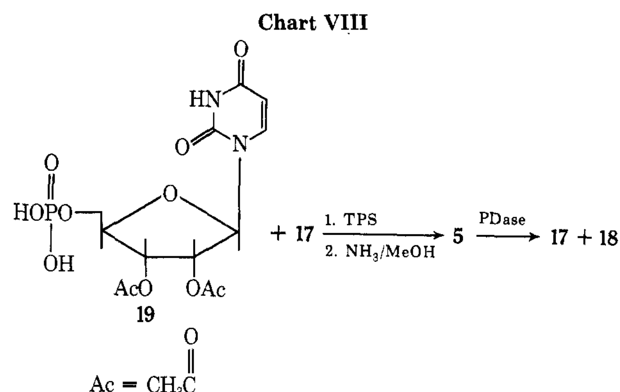
**Chart VII**




Prolonged treatment (20 hr) of 18 with the reagent 15 afforded a mixture of 5 and 1-oxido-2-pyridylmethyl-3-(1-oxido-2-pyridylmethyl)uridine 5′-phosphate (6) in 85% total yields. Separation of 6 and 5 could be achieved by a DEAE-cellulose column chromatography.

The structure confirmation of 6 was carried out as follows. Hydrolysis of 6 either with a mixture of snake venom phosphodiesterase and alkaline phosphomonoesterase or initially with the phosphodiesterase and subsequently with the phosphomonesterase afforded 3-(1-oxido-2-pyridylmethyl)uridine (20) (Chart IX) which was found to be identical with a sample which had been prepared

**Chart VIII**




according to a route in Chart X: 2′,3′-*O*-isopropylideneuridine (21) was treated with 1-oxido-2-pyridylmethyl chloride (22)[12] in the presence of potassium carbonate in DMF for 3 hr to give 2′,3′-*O*-isopropylidene-3-(1-oxido-2-pyridylmethyl)uridine (23). Deacetonization with refluxing 20% aqueous acetic acid afforded an authentic sample of 20.

**Chart IX**

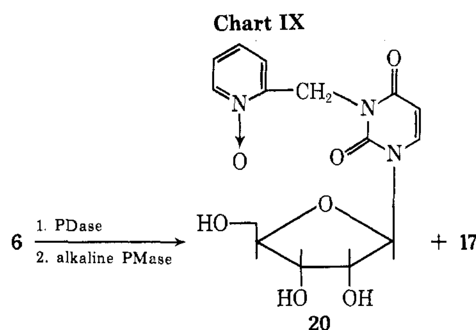
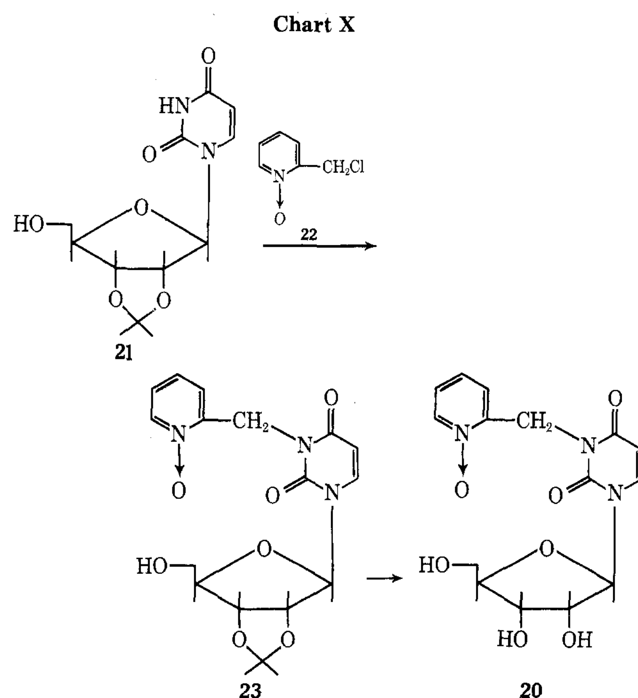


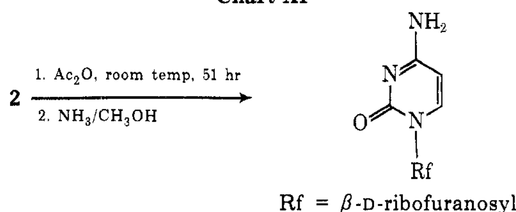

**Chart X**




Deblocking of the 1-oxido-2-pyridylmethyl group of 2 was achieved by treatment with acetic anhydride at room temperature for 51 hr, followed by methanolic ammonia treatment (Chart XI). Recovery of cytidine was 86.7%.

Deblocking of nucleotides 4 and 6 could be achieved in 96 and 86% yields, respectively, by treatment of the nucleotides with acetic anhydride at 60° for 35 hr (in the

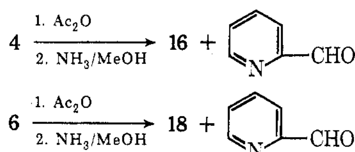
Chart XI




Rf = $\beta$-D-ribofuranosyl

case of 4) and at 37° for 72 hr (in the case of 6) and by subsequent methanolic ammonia treatment (Chart XII).

Chart XII




Thus it was found that the 1-oxido-2-pyridylmethyl group was quite useful for the protection of nucleosides and nucleotides. It is worthy of note that our new reagent (15) was found to be capable of introduction of op protection into hydroxyl functions of the phosphate of the nucleotides even in aqueous solution.

As a logical extention of the present investigation, we are trying to apply this protection for the synthesis of the oligoribonucleotides.

## Experimental Section

**General.** The ultraviolet spectra were determined using a Hitachi recording spectrophotometer (Model 3T) and gas chromatography was carried out by a Shimazdu gas chromatograph (Model GC-4-APF). Infrared spectra were taken on an infrared spectrophotometer (DS-701G) in KBr tablets. Nuclear magnetic resonance (nmr) spectra were determined with a Hitachi high-resolution nmr spectrometer (Model R24) in deuteriochloroform. The chemical shifts were reported in parts per million downfield from tetramethylsilane as internal standard. Snake venom 5′-nucleotidase (*Clotalus adamanteus*) was obtained from Sigma Chemical Co. Digestion with this enzyme was carried out as reported.[16] Snake venom phosphodiesterase was obtained from Worthington Biochemicals Co. and was dissolved 1 mg in 1 ml. This solution was used for the enzymatic digestion.

Paper electrophoresis (PEP) was performed on Toyo-Roshi paper No. 51A (45 × 10 cm) impregnated with 0.05 *M* triethylammonium bicarbonate (TEAB, pH 8.0) using 700 V or with 0.05 *M* acetate buffer (pH 3.7) using 1000 V conducted on flat-bed apparatus. Paper chromatography was carried out by the ascending technique on Toyo-Roshi paper No. 51A using the following systems: solvent A, *n*-BuOH-AcOH-$H_2O$ (5:2:3); solvent B, *i*-PrOH-$NH_4OH$-$H_2O$ (7:1:2). DEAE cellulose refers to the product of Jujo Seishi Co. and a gift therefrom which was used in the bicarbonate form. Silica gel for the column chromatography refers to Kieselgel 60 (Merck). Silica gel for the thin layer chromatography (tlc) refers to Kieselgel HF 254 (Merck). Two-dimensional tlc on Avicel SF plate (10 × 10 cm) was performed with solvent A and then with solvent B. In each case, about 1–2 $A_{260\,nm}$ units of nucleotides were used. Extraction and estimation of each spot were carried out as reported.[17] The following molar extinction coefficients were used: 1-oxido-2-pyridylmethyladenosine 5′-phosphate (4), 25,000; 1-oxido-2-pyridylmethyluridine 5′-phosphate (5), 18,500; and 1-oxido-2-pyridylmethyl-3-(1-oxido-2-pyridylmethyl)-uridine 5′-phosphate (6), 27,000.

Unless otherwise specified, the solvent was removed under reduced pressure (with a water aspirator) with a rotating evaporator.

The melting points are uncorrected. Elemental analyses were performed by a staff of the analytical laboratory in the Faculty of Pharmaceutical Sciences, Hokkaido University.

**1-Oxido-2-pyridylmethyl Chloride HCl (22). Improved Method.** In a three-necked flask, 1-oxido-2-pyridylcarbinol (17, 10.0 g) was dissolved in 100 ml of chloroform. There was added dropwise 12.6 g (7.7 ml, 1.1 equiv) of freshly distilled thionyl chloride at 0° in 2 hr. After the addition was complete, the mixture was heated at 50–60° (bath temperature) for 2 hr. The reaction mixture was allowed to come to room temperature. Ethanol (0.5 ml) was added to decompose excess thionyl chloride. The reaction mixture was then concentrated to dryness. The residue was crystallized from acetone, mp 105–109°, yield 12.39 g (86.6%).

**1-Oxido-2-pyridylmethylamine Hydrochloride (9).** Crude product (without crystallization) prepared from 7.3 g of 17 was dissolved in 300 ml of saturated ammonium hydroxide (0°). The solution was kept at room temperature overnight and then concentrated to dryness. The residue was crystallized from 120 ml of absolute ethanol. The first crop weighed 5.03 g. After concentration, the mother liquor gave 1.0 g of 9, total yield 6.03 g (58%), mp 114–115°.

*Anal.* Calcd for $C_6H_9N_2OCl$: C, 44.87; H, 5.79; N, 16.92; Cl, 22.08. Found: C, 44.69; H, 5.70; N, 17.10; Cl, 22.07.

**1-Oxido-2-pyridylmethylamine.** 1-Oxido-2-pyridylmethylamine hydrochloride (9, 1.8 g) was neutralized with a resin (Dowex 1, $OH^-$ form). After filtration, the filtrate was concentrated to dryness. Crystallization from ethyl acetate–acetonitrile–ethyl ether (1:1:1) gave the analytical sample, yield, 1.0 g, mp 82–83°.

*Anal.* Calcd for $C_6H_8N_2O$: C, 58.06; H, 6.45; N, 22.58. Found: C, 57.94; H, 6.42; N, 22.35.

**$N^6$-(1-Oxido-2-pyridylmethyl)adenine (1).** 6-Methylthiopurine (3.2 g) was oxidized with saturated aqueous bromine solution (150 ml) for 30 min.[9] The solution was neutralized with a resin (Dowex 1 $OH^-$ form, 53 ml). The resin was filtered off. The filtrate was concentrated to dryness. The residue (1.4 g) was dissolved in aqueous methanol (1:1) and then treated with 1-oxido-2-pyridylmethylamine prepared from 0.79 g of the chloride 22. The solution was heated for 1 hr and then concentrated to dryness. The residue was twice recrystallized from water: mp 235–237° dec; yield 0.93 g (20%); uv $\lambda_{max}$ ($H_2O$) 260 nm ($\epsilon$ 25,000), $\lambda_{max}$ (pH 1) 260, 275 nm (sh), $\lambda_{max}$ (pH 11) 270 nm.

*Anal.* Calcd for $C_{11}H_{10}N_6O$: C, 54.54; H, 4.16; N, 34.69. Found: C, 54.30; H, 4.15; N, 34.49.

**Oxidation of 4-Thiouridine (7) and Syntheses of $N^4$-(1-Oxido-2-pyridylmethyl)cytidine (2).** To a solution of 4-thiouridine[8] (7, 1.0 g, 380 mmol) in 80 ml of phosphate buffer (pH 7.0) was added at 0° 1 equiv of 0.1 *M* potassium permanganate solution. The mixture was kept at the same temperature for 15 min.[7] Manganese dioxide formed was removed by centrifugation. One equivalent of 9 was added to the filtrate. The solution was adjusted to pH 8.5 with 0.5 *M* potassium hydroxide and stored for 25 hr at room temperature. After checking that the reaction was complete by uv, the mixture (which contained 99% crude yield of 2) was treated with activated charcoal (Shirasagi brand, 2.5 g). The charcoal was collected by filtration, washed with water, extracted with 50% aqueous ethanol containing 2% ammonia, and filtered. The filtrate was concentrated to dryness and recrystallized from water, mp 202–205°, yield 0.326 g (34.7%).

*Anal.* Calcd for $C_{15}H_{18}N_4O_6$: C, 51.42; H, 5.18; N, 15.99. Found: C, 51.04; H, 5.21; N, 16.05.

**$N^6$-(1-Oxido-2-pyridylmethyl)adenosine (3).** To a solution of 9-($\beta$-D-ribofuranosyl)-6-ethylthiopurine (12, 1.21 g, 3.87 mmol) in 260 ml of phosphate buffer (pH 7.0) was added 1 equiv of saturated aqueous bromine solution. After checking that bromine had been completely consumed with an iodine-iodide-starch paper, another 1 equiv of bromine solution was added. The solution was kept at room temperature overnight. There was then added 0.58 g (1 equiv) of 9. The solution was adjusted to pH 8.5 with 2 *N* sodium hydroxide and kept at ambient temperature for 24 hr. The solution was concentrated to dryness. The residue was dissolved in methanol and filtered. The filtrate was concentrated to dryness. The residue was again dissolved in 3 ml of methanol and applied to a silica gel column (weight of silica gel, 80 g). The column was washed with 2 l. of $CHCl_3$-MeOH (7:1). Fractions containing 3 were collected and concentrated to dryness (850 mg). The residue was rechromatographed in a similar way, except that 30 g of silica gel was used and the column was washed with $CHCl_3$-MeOH (5:1). Fractions containing 3 were collected and concentrated to dryness. The residue was crystallized from water: mp 182–185° dec; yield 286 mg (20%); uv $\lambda_{max}$ ($H_2O$) 260 nm, $\lambda_{max}$ (pH 1) 260 nm (275 nm, sh), $\lambda_{max}$ (pH 11) 260 nm (270 nm, sh).

**Acid Hydrolysis of $N^6$-(1-Oxido-2-pyridylmethyl)adenosine (3).** Compound 3 (10 mg) was dissolved in 0.1 *N* HCl (2 ml). The solution was heated under reflux for 1 hr. $R_f$ values of the hydrolysate (paper chromatography, solvent A and B) were 0.63 and 0.75, respectively, which were found to be identical with the re-

spective $R_f$ value of **1**. Uv of the extracts of each spot was similar to that of **1**.

***p*-Tosylhydrazone of 2-Formylpyridine 1-Oxide.** 2-Formylpyridine 1-oxide[10] prepared by selenium dioxide oxidation of 250 g of $\alpha$-picoline was dissolved in 1 l. of methanol. This solution was treated with 500 g of *p*-tosylhydrazine dissolved in 1 l. of methanol to give 470 g of product (79.4%), mp 135–137°.

*Anal.* Calcd for $C_{13}H_{13}N_3O_3S$: C, 53.61; H, 4.46; N, 14.43; S, 11.00. Found: C, 53.49; H, 4.50; N, 14.29; S, 10.98.

**1-Oxido-2-pyridyldiazomethane (15, Chloroform Solution).** The *p*-tosylhydrazone of 2-formylpyridine 1-oxide (10 mmol) was dissolved in 40 ml of ethanolic sodium ethoxide prepared from the equivalent of sodium. The solution was heated at 50° for 20 min and then concentrated to dryness. The residue was dissolved in 50 ml of chloroform. Insoluble material (sodium *p*-toluenesulfinate) was filtered off. The filtrate was employed for alkylation. Glc (column: silicone ov-1 on Chromosorb, column temperature 150°, detector temperature 215°) showed that the above solution contained 2-ethoxymethylpyridine 1-oxide (*ca.* 10%) in addition to **15**. The ether was isolated by preparative tlc: mp of the picrate 125° (crystallized from methanol–ethyl ester); yield 280 mg (10%); ir (neat) 2080 ($N{\equiv}N^+$), 1235 cm$^{-1}$ ($^+N\text{-}O^-$).

*Anal.* Calcd for $C_{14}H_{14}N_4O_9$: C, 44.00; H, 3.69; N, 14.65. Found: C, 44.02; H, 3.70; N, 3.66.

**Determination of Concentration of 15 in Chloroform Solution.** Metallic sodium (870 mg) was dissolved in 50 ml of dry ethanol. To the solution was added 11 g of the *p*-tosylhydrazone of 2-formylpyridine 1-oxide in portions. The solution was refluxed for 1 hr and then concentrated to dryness. The residue was dissolved in 50 ml of chloroform. Insoluble material was filtered off. There was then added 4.7 g of benzoic acid to the filtrate. The solution was kept at room temperature for 3 hr and then concentrated to dryness. The residue was triturated with 50 ml of 10% $NaHCO_3$. Product was collected by filtration, yield 4.1 g (47%, based on the *p*-tosylhydrazone), mp 125–126°.[5] Based on the assumption that the alkylation was quantitative, the yield of **15** could be estimated to be 47%. The above chloroform solution had contained *ca.* 5.13 g of **15**.

**General Procedure for Alkylation with 15 (Alkylation of Benzoic Acid as a Representative).** To a chloroform solution (30 ml) of 1-oxido-2-pyridyldiazomethane (**15**) prepared from 2.99 g of the *p*-tosylhydrazone and 0.23 g of sodium was added a DMF solution (30 ml) of benzoic acid (1.0 g). The solution was kept at room temperature for 3 hr and then concentrated to half of its volume, and water was added. Crystals deposited were collected and recrystallized from ethanol, yield 1.02 g (quantitative).[5a]

**1-Oxido-2-pyridylmethyladenosine 5′-Phosphate (4).** To a suspension of adenosine 5′-phosphoric acid (**16**, 46.2 mg) in 10 ml of water was added sodium hydrogen carbonate solution until solution resulted. There was then added a chloroform solution (20 ml) of **15** prepared from 1.98 g of the *p*-tosylhydrazone. The solution was kept at room temperature overnight. After making sure that the reaction was complete (by paper electrophoresis), the aqueous layer was extracted with three 10-ml portions of chloroform and separated. The aqueous layer was applied to a DEAE-cellulose column (1.6 × 40 cm). The column was initially washed with 500 ml of water and then with a linear gradient of 500 ml of water and 500 ml of 0.2 *M* TEAB solution. The effluent was monitored at 260 nm. Ten grams of effluent was collected as one fraction. Fractions 14–27 were pooled and rechromatographed under similar conditions: fractions 15–23 (fraction 1a) were colored and discarded; fractions 24–29 containing **4** (1b) were pooled and concentrated. PEP examination of fraction 1b showed the presence of a single spot corresponding to **4**, which showed a positive reaction against a metaperiodate–benzidine spray reagent:[15] uv $\lambda_{max}$ (pH 2) 257 nm, $\lambda_{max}$ ($H_2O$) 258 nm [$\epsilon$ (p) 25,000];[14] yield 90%.

**Structural Confirmation of the Product 4.** Fraction 1b (50 $\mu$l, 15 $A_{260\,nm}$ units) and 30 $\mu$l of 0.1 *M* TEAB solution were mixed and adjusted to 100 $\mu$l with distilled water. The solution was incubated with 20 $\mu$l of snake venom phosphodiesterase solution at 37° overnight. Electrophoretic examination (pH 8.0) of the reaction mixture showed the presence of adenosine 5′-phosphate (**16**) and 1-oxido-2-pyridylcarbinol (**17**) in a molar ratio of 1:1.

**1-Oxido-2-pyridylmethyluridine 5′-Phosphate (5).** Uridine 5′-phosphate (**18**, disodium salt, $2.5H_2O$, 300 mg, 0.606 mmol) was converted to the free acid with Dowex 50Wx8 ($H^+$ form). The solution (10 ml) of the free acid was mixed with a chloroform solution (20 ml) of **15** which had been prepared by the above general procedure. The mixture was kept stirring for 20 min at room temperature. The solvent was removed. The residue was dissolved in a small amount of water. The solution was applied to a DEAE-cellulose column (3.5 – 30 cm). The column was initially washed with 100 ml of water and then with 0.06 *M* TEAB solution. Fractions containing **5** were collected and adjusted to pH 4 with a resin (Dowex 50Wx8, $H^+$ form). The solution was concentrated to dryness at 30°, yield 9800 $A_{260\,nm}$ units (0.534 mmol), 89%.

**Structural Confirmation of the Product (5).** An incubation mixture contained 30 $A_{260\,nm}$ units of **5**, 20 $\mu$l of snake venom phosphodiesterase, and 100 $\mu$l of 0.3 *M* triethylammonium bicarbonate solution. The mixture was incubated at 37° for 15 hr. Nucleotide **5** was completely hydrolyzed and the reaction mixture contained uridine 5′-phosphate (**18**) and 1-oxido-2-pyridylcarbinol (**17**) in a molar ratio of 1:1. Uv spectral properties and electrophoretic mobilities (at pH 3.7 as well as at pH 8.0) of **5** were identical with those of an authentic sample of 1-oxido-2-pyridylmethyluridine 5′-phosphate (**5**), prepared as described below.

**Alternative Synthesis of 1-Oxido-2-pyridylmethyluridine 5′-Phosphate (5). Synthesis of 2′,3′-Di-*O*-acetyluridine 5′-Phosphate (19).** To a solution of uridine 5′-phosphate (disodium salt $H_2O$, 3.7 g, 7.62 mmol, determined spectrophotometrically) in 50 ml of pyridine was added acetic anhydride (20 ml). The mixture was kept in the dark at room temperature for 6 days. A clear solution resulted. There was then added methanol (25 ml) at 5° and then the mixture was allowed to come to 30°. The solution was concentrated to dryness. The residue was dissolved in 40% aqueous pyridine (50 ml). The solution was allowed to stand at room temperature for 12 hr. The solvent was removed. The residue was triturated with ethanol (100 ml) to yield a white powder, which was dried over phosphorus pentoxide at 60°. Tlc examination showed the presence of a new spot (which gave a negative reaction against a metaperiodate–benzidine spray reagent).[15] Uv spectra were quite similar to those of **18**, yield 3.2 g (84.5%, calculated as disodium salt $2.5H_2O$).

**Synthesis of 5.** 1-Oxido-2-pyridylcarbinol (**17**, 125 mg, 1 mmol) and nucleotide **19**[13] (412 mg, 1 mmol) were dissolved in DMF (5 ml). The solvent was removed *in vacuo* (1 mm). The completely dried residue was dissolved in DMF (4 ml). There was then added TPS (604 mg, 2 mmol). The solution was kept at room temperature for 24 hr. Cold water (3 ml) was added to the reaction mixture at 5°. The solution was allowed to come to room temperature and was kept at the same temperature for 1 hr. Tri-*n*-butylamine (1.5 ml) was added. The mixture was kept at room temperature for 30 min and filtered. The filtrate was treated with three 30-ml portions of ether. The aqueous layer was concentrated to dryness. Paper electrophoresis showed the presence of a single spot whose mobility was different from that of **17** and **18**. The reaction mixture was then applied to a DEAE-cellulose column (1.2 × 40 cm, fraction volumn 11 ml). The column was washed with a linear gradient of 500 ml of water and 500 ml of 0.3 *M* TEAB. Fractions 11–19 were pooled (4.400 $A_{260\,nm}$ units). A portion (35 $A_{260\,nm}$ units) was concentrated to dryness. The residue was dissolved in methanolic ammonia (1 ml). The solution was kept at room temperature for 20 hr. The mixture was concentrated to dryness, electrophoretic mobility $R_{5'UMP}$ (pH 3.7) 1.01. Enzymatic hydrolysis of this sample with snake venom phosphodiesterase showed that this nucleotide was completely hydrolyzed to uridine 5′-phosphate (**18**) and **17**. The rest of the mixture was similarly treated; 1-oxido-2-pyridylmethyluridine 5′-phosphate (**5**) was obtained in a yield of 70%. This sample was used as an authentic sample of **5** for the above-mentioned comparison.

**1-Oxido-2-pyridylmethyl-3-(1-oxido-2-pyridylmethyl)uridine 5′-Phosphate (6).** To a chloroform solution (30 ml) of **15** prepared from 2.83 g of the *p*-tosylhydrazone of 2-formylpyridine 1-oxide was added an aqueous solution (10 ml) of uridine 5′-phosphate (**18**, disodium salt, 133.6 mg, 1000 $A_{260\,nm}$ units). The solution was kept at room temperature overnight. Paper electrophoretic examination (at pH 8.0) of the reaction mixture showed the absence of the starting material **18**. The aqueous layer was separated and washed with three 10-ml portions of chloroform and was then applied to DEAE-cellulose column chromatography (1.6 × 40 cm, fraction size 10 ml). The column was washed with a linear gradient of 500 ml of water and 500 ml of 0.2 *M* TEAB solution. Fractions 32–48 (which are referred to as fraction 1) and fractions 49–59 (fraction 2) were separately pooled. Fraction 1 contained products (**5** and **6**), whereas fraction 2 contained a small amount of the starting material (**18**). Fraction 1 was rechromatographed under similar conditions. On chromatogram, two peaks (1a and 1b) appeared: 1a, fractions 22–29; 1b, fractions 35–40. Total optical density (TOD) in fraction 1a was 1000 $A_{260\,nm}$ units; TOD in fraction 1b was 500 $A_{260\,nm}$ units. Electrophoretic mobilities of nucleotides in fraction 1a and 1b were $R_{5'UMP}$ 0.52 and 0.67 (at pH 8.0), respectively. On the basis of

these relative electrophoretic mobilities and the spectral data it was tentatively concluded that fraction 1a and 1b contained bis-op-protected uridine 5′-phosphate (**6**) and op-protected uridine 5′-phosphate (**5**), respectively.

**Confirmation of the Structure of Nucleotides in Fraction 1a and 1b. Enzymatic Hydrolysis of Nucleotides 6 in Fraction 1a.** An incubation mixture contained 30 $A_{260\,nm}$ units of fraction 1a (50 μl), 40 μl of 0.14 *M* TEAB solution, 40 μl of snake venom phosphodiesterase, and 40 μl of alkaline phosphomonoesterase. The mixture was incubated at 37° for 20 hr.

PEP (pH 8.0) and paper chromatographic (solvent A) examination showed that **6** in fraction 1a was completely hydrolyzed with these enzymes to 1-oxido-2-pyridylcarbinol (**17**), inorganic phosphate, and 3-(1-oxido-2-pyridylmethyl)uridine (**20**). The structure of **20** was unequivocally established by comparison with a sample which was prepared by an unambiguous synthesis.

The structure of **5** in fraction 1b was determined as described before.

**3-(1-Oxido-2-pyridylmethyl)-2′,3′-*O*-isopropylideneuridine (23).** 2′,3′-*O*-Isopropylideneuridine (**21**, 2.85 g, 10 mmol) was treated with 1-oxido-2-pyridylmethyl chloride HCl (**22**, 1.8 g, 12.4 mmol) in the presence of dried, powdered $K_2CO_3$ (4.0 g) in DMF (25 ml) at room temperature for 36 hr. The reaction mixture was then filtered. The filtrate was concentrated to dryness. The residue was triturated with ether and filtered. Recrystallization from aqueous acetone (30 ml, 5:1) afforded the analytical sample: yield 3.20 g (80%); mp 204–205°; uv $\lambda_{max}$ (0.1 *N* HCl) 256.5 nm, $\lambda_{max}$ ($H_2O$) 256.0 nm, $\lambda_{max}$ (0.1 *N* NaOH) 255 nm. The elemental analysis and nmr were expected for **23**: nmr (TMS external standard, DMSO-$d_6$) 1.30 (s, 3, $CH_3$ of isopropylidene), 1.5 (s, 3, $CH_3$ of isopropylidene), 3.3 (s, 2, 5′-$CH_2$), 4.2 (m, 1, 5′-OH), 5.9 (d, 1, anomeric proton), multiplet centered around 7.3 (m, 3, pyridine), 8.0 ppm (d, 1, 6-H), absence of signals downfield from 9.0 ppm.

*Anal.* Calcd for $C_{18}H_{21}N_3O_7$: C, 55.24; H, 5.41; N, 10.74. Found: C, 55.52; H, 5.42; N, 10.49.

**3-(1-Oxido-2-pyridylmethyl)uridine (20).** A solution of **23** (780 mg, 2.0 mmol) in 20% aqueous acetic acid was refluxed for 2 hr. The solution was concentrated to dryness. The residue was recrystallized from ethanol: mp 186–187°; yield 630 mg (90%); $R_f$ (solvent B) 0.69; nmr (TMS external standard, DMSO-$d_6$) 3.65 (s, 2, 5′-$CH_2$), 5.04 (s, 2, methylene of α-picolyl), 5.80 (d, 1, anomeric proton), 5.88 (d, 2, 5-H), 7.30 (m, 3, pyridine), 8.20 (d, 1, 6-H); uv $\lambda_{max}$ (0.1 *N* HCl) 256 nm (ε 18,500), $\lambda_{max}$ ($H_2O$) 256.5 nm (ε 18,500), $\lambda_{max}$ (0.1 *N* NaOH) 256 nm (ε 18,500).

*Anal.* Calcd for $C_{15}H_{17}N_3O_7$: C, 51.27; H, 4.88; N, 11.96. Found: C, 51.14; H, 4.81; N, 11.74.

**Deblocking. Conversion of $N^4$-(1-Oxido-2-pyridylmethyl)cytidine to Cytidine.** $N^4$-(1-Oxido-2-pyridylmethyl)cytidine (**2**, 100 mg, 0.286 mmol) was dissolved in acetic anhydride (10 ml). The solution was allowed to stand with stirring at 30° for 51 hr. The solution was concentrated to dryness. The residue was dissolved in methanolic ammonia in a stoppered vessel. The solution was kept at room temperature overnight. The mixture was concentrated to dryness. The residue was dissolved in a small amount of water. The aqueous solution was applied to a Dowex 1x8 column (1.2 × 32 cm formate form, fraction size 15 ml). The column was initially washed with water (750 ml) and then with 0.2 *M* formic acid (500 ml). The effluent was monitored at 260 nm. Fractions containing cytidine were pooled and concentrated to dryness. Recovery of cytidine was 0.248 mmol (86.7%). This sample was found to be identical with cytidine on the criteria of uv spectra and $R_f$ value in solvent systems A and B.

**Conversion of 1-Oxido-2-pyridylmethyl-3-(1-oxido-2-pyridylmethyl)uridine (6) to Uridine 5′-Phosphate (18).** To a solution of **6** (triethylammonium salt, 991 $A_{260\,nm}$ units) in DMF (20 ml) was added acetic anhydride (50 ml). The mixture was kept at 37° for 72 hr. The solution was concentrated to dryness below 40°. The residue was dissolved in methanol (60 ml) saturated with ammonia. The mixture was kept at room temperature overnight. The solvent was removed. The residue was dissolved in a small amount of water. The aqueous solution was applied to a DEAE-cellulose column (0.7 × 40 cm). The column was initially washed with 200 ml of water (the effluent was discarded) and then with 0.2 *M* TEAB solution (200 ml). Fractions containing **18** were collected, concentrated to dryness, and lyophilized, PEP $R_{5'UMP}$ 1.08 (pH 8.0). Uv spectra were identical with those of authentic 5′-UMP, yield 229 $A_{260\,nm}$ units (86%).

**Conversion of 1-Oxido-2-pyridylmethyladenosine 5′-Phosphate (4) to Adenosine 5′-Phosphate (16).** Nucleotide **4** (triethylammonium salt, 6200 $A_{260\,nm}$ units) was dissolved in 5 ml of acetic anhydride. The solution was heated at 60° for 35 hr. The solution was then concentrated to dryness below 40°. The residue was dissolved in 10 ml of methanol saturated with ammonia at 0°. The solution was kept at room temperature overnight. The solvent was removed. The residue was applied to a DEAE-cellulose column (3 × 40 cm). The column was washed with 500 ml of water and then with a linear gradient of 0.2 *M* tetraethylammonium bicarbonate solution (1.5 l.) and water (1.5 l.), fraction size 18 g. Fractions containing **16** (fractions 59–85) were concentrated to dryness and lyophilized, PEP $R_{5'AMP}$ 1.00 (pH 8.0), $R_f$ (EtOH-1 *M* $AcONH_4$, 1:1) 0.41. Uv spectra were identical with those of an authentic sample of 5′-AMP, yield 3560 $A_{260\,nm}$ units (96%).

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**Registry No.**—**1**, 50921-44-3; **2**, 51022-68-5; **3**, 50921-80-7; **4**, 50908-30-0; **5**, 50921-81-8; **6**, 50908-28-6; **7**, 13957-31-8; **9**, 50921-45-4; **9** hydrochloride, 50921-46-5; **12**, 13286-04-9; **15**, 50908-23-1; **16**, 61-19-8; **17**, 10242-36-1; **18**, 58-97-9; **19**, 48215-95-8; **20**, 50908-29-7; **21**, 362-43-6; **22** hydrochloride, 20979-34-4; **23**, 50921-79-4; 6-methylthiopurine, 50-66-8; 2-formylpyridine 1-oxide, 7216-40-2; 2-formylpyridine 1-oxide *p*-tosylhydrazone, 50908-22-0; 2-ethoxymethylpyridine 1-oxide picrate, 21901-67-7.

## References and Notes


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(2) A part of the present work has appeared in preliminary form.[5] This paper was also presented in part at the 4th International Congress of Heterocyclic Chemistry, Salt Lake City, Utah, July 1973.

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